Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 5557–5561, September 1982
Cell Biology

The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border

(experimental glomerulonephritis/nephritogenic antigen/immunocytochemistry)

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Communicated by George E. Palade, May 27, 1982

ABSTRACT **Purified brush border fractions prepared from rat kidneys were solubilized in detergent, iodinated, and subjected to immunoprecipitation to identify the pathogenic antigen present in brush border membranes that is responsible for the production of Heymann nephritis (HN). Purified IgG prepared from the sera of rabbits or rats immunized with a crude cortical preparation, known as FxlA, precipitated multiple peptides, whereas IgG eluted from glomeruli of rats with active or passive HN specifically immunoprecipitated a single large glycoprotein (M_r = 330,000). This protein (gp330) was subsequently purified by gel filtration and lentil lectin affinity chromatography from detergent-solubilized brush border membranes. When rats were immunized with purified gp330, they developed anti-brush border antibodies and active HN. IgG prepared from the serum of rats with active HN caused passive HN when injected into normal recipients. Rats immunized against brush border membrane proteins depleted of gp330 developed anti-brush border antibodies but did not develop HN. Further analysis of gp330 indicated that it is solubilized by detergent treatment of isolated brush border microvilli, and its antigenic component is released from intact microvilli by trypsin. By immunoperoxidase staining it was localized to the luminal side of the brush border membranes. These results indicate that (*i*) gp330 is the pathogenic antigen of HN; (*ii*) the antigen is a glycoprotein of the brush border membrane; and (*iii*) it is disposed with its pathogenic domain(s) facing the tubule lumen.**

Heymann nephritis (HN) is an experimental glomerulonephritis in rats that is of interest in renal pathology because it closely resembles membranous glomerulonephritis, a common type of human glomerular disease. HN is induced by immunizing rats with kidney extracts (1), a crude homologous cortical fraction known as FxlA (2), or extracts of brush border fractions derived from the proximal kidney tubule (3). Immunized animals develop circulating antibodies that are deposited in the lamina rara externa of the glomerular basement membrane (GBM); identical patterns are seen either after immunization with FxlA [active HN (2)] or when serum or purified IgG from immunized animals is injected into normal recipients [passive HN (4, 5)]. Immunofluorescence studies clearly indicate that the circulating antibodies crossreact with components of the brush borders of the proximal kidney tubule (6, 7), but the identity of the antigen responsible for the deposition of immune complexes in the GBM is still unknown. It has been variously claimed to be a lipoprotein (2) or a glycoprotein (3, 8, 9) present in the membrane of the brush border of the proximal tubule.

We here report experiments in which we have isolated the pathogenic antigen of HN and have identified it as a brush border membrane glycoprotein of M_r 330,000 (gp330).

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MATERIALS AND METHODS

Materials. Mannitol, α-methylmannoside, pepstatin A, antipain, and β-galactosidase were purchased from Sigma; lactoperoxidase, glucose oxidase, sodium deoxycholate (DOC), benzamidine, and diisopropyl fluorophosphate from Calbiochem; carrier-free $Na^{125}I$ and Triton X-100 from New England Nuclear; *N*-tosylphenylalanine chloromethyl ketone (TPCK)-trypsin and lima bean trypsin inhibitor from Worthington; acrylamide, sodium dodecyl sulfate, molecular weight standards, Bio-Gel A-5m (100–200 mesh), and DEAE Bio-Gel from Bio-Rad; Protein A-Sepharose CL-4B and lentil-Sepharose 4B from Pharmacia; pertussis vaccine from Eli Lilly; complete Freund adjuvant from Difco; rabbit anti-rat IgG, rabbit anti-rat albumin IgG, and rhodamine-labeled rabbit anti-rat IgG from Cappel; and sheep horseradish peroxidase-conjugated Fab anti-rabbit Ig from the Institute Pasteur.

Isolation of Kidney Brush Border Microvilli. Kidneys of male Sprague–Dawley rats (250 g; Charles River) were perfused via the abdominal aorta with ice-cold Tris-buffered saline (Tris/NaCl) containing 150 mM NaCl, 10 mM Tris·HCl at pH 7.2, 1 mM pepstatin A, 1 mM antipain, and 2 mM benzamidine. The cortices were removed and purified microvilli were prepared by using 10 mM $CaCl_2$ as a precipitating agent (10). The purity of the final microvillus suspension was assessed by electron microscopy and by the relative enrichment of alkaline phosphatase activity (11). Aliquots of the suspension were stored in liquid nitrogen or used directly for lactoperoxidase-mediated radioiodination (12). Usually 1 mg of microvillus protein [determined by the Lowry method (13)] was recovered from 1 g of kidney cortex.

Solubilization of Microvillus Membrane Proteins and Separation by Gel Chromatography. Isolated microvilli were extracted in DOC buffer (1% DOC/50 mM Tris·HCl, pH 8.6, supplemented with 1 mM diisopropyl fluorophosphate) for 20 min at room temperature; 1 ml of DOC buffer was used per mg of microvillus protein. The lysate was centrifuged at 38,000 × *g* for 30 min; the resultant supernatant was concentrated to 3 ml by ultrafiltration on an Amicon PM-10 membrane, loaded on a 100 × 1.5 cm Bio-Gel A-5m column (100–200 mesh), and run at 8 ml/hr with DOC buffer containing 0.05% sodium azide at 20°C. Fractions were monitored for protein content by reading the OD_{280} or by determining the cpm in a gamma counter. The composition of the column fractions was monitored by $NaDodSO_4$/polyacrylamide gel electrophoresis.

Affinity Chromatography on Lentil Lectin-Sepharose CL-4B. Fractions obtained from the Bio-Gel A-5m column, after

Abbreviations: HN, Heymann nephritis; DOC, deoxycholate; TPCK, *N*-tosylphenylalanine chloromethyl ketone; FxlA, crude rat renal cortical extract; Tris/NaCl, Tris-buffered saline; GBM, glomerular basement membrane; kDal, kilodalton(s).

the void peak, were pooled, concentrated to 5–6 ml (on an Amicon PM-10 membrane), and loaded on a lentil-Sepharose CL-4B column. After removal of unbound material, 50 bed vol of DOC buffer and 20 bed vol of 20 mM α-methylmannoside in DOC buffer were passed through the column until the OD_{280} or the cpm readings (or both) reached baseline values. The lentil-Sepharose was then eluted by recycling 5 bed vol of DOC buffer containing 10 mM EDTA, 6 M urea, and 500 mM α-methylmannoside for 20 min. With this protocol, typically >90% of the bound material was released. The composition of the fractions was determined by $NaDodSO_4$/polyacrylamide gel electrophoresis.

Immunization of Rabbits with FxlA. For the production of heterologous anti-FxlA IgG, a rabbit was immunized with 10 mg of FxlA in complete Freund adjuvant injected in the rear foot pads and boosted 3 wk later with 5 mg of FxlA in incomplete Freund adjuvant.

Induction of Active HN in Rats. FxlA was prepared from pooled frozen kidneys of 50 rats as described by Edgington *et al.* (2). Rats were immunized with 7–10 mg of the lyophilized FxlA suspended in complete Freund adjuvant and were injected into the rear foot pads.

Induction of Passive HN in Rats. Passive HN was induced in rats by intravenous injection into the tail vein of either 5 mg of purified IgG or 500–700 μl of serum obtained from immunized rabbits or from rats with active HN. After 3–5 days the kidneys of the rats were perfused with Tris/NaCl and processed for immunofluorescence microscopy.

Purification of Serum IgG and Elution of IgG from Glomeruli. IgG of rabbits and rats was purified by ammonium sulfate precipitation and ion exchange chromatography on DEAE Bio-Gel A (14). Glomeruli were isolated and purified by the sieving method of Krakower and Greenspon (15). IgG was eluted from the isolated glomeruli by extraction with 20 mM citrate buffer at pH 3.2 at 37°C for 30 min (16) and was used for indirect immunofluorescence microscopy or immune precipitations.

Induction of HN with Purified gp330. For the induction of active HN, six male Fischer rats (250 g; Charles River) were each injected into the rear foot pads with 60 μg of lectin-purified gp330 suspended in 100 μl of Tris/NaCl and 100 μl of complete Freund adjuvant. In addition, 50 μl of pertussis vaccine suspension was injected subcutaneously into the dorsal skin of the rear foot pads. Sera were obtained at weekly intervals after the 4th week, and the appearance of anti-brush border antibodies was monitored by indirect immunofluorescence microscopy on normal rat kidney frozen sections.

For the induction of passive HN, either 500 μl of serum or 3–5 mg of purified IgG of rats immunized against gp330 was injected intravenously into normal Sprague–Dawley rats. Kidneys were perfused and prepared for immunofluorescence 3–4 days later.

Control Immunizations. As a positive control, three Fischer rats were immunized against 3 mg of isolated kidney brush-border microvilli suspended in complete Freund adjuvant (3). As negative controls, two Fischer rats were immunized with 300–500 μg of protein from the Bio-Gel A-5m column fractions that contained microvillus components depleted of gp330. Two Fischer rats were injected only with complete adjuvant emulsified in Tris/NaCl. After 6–8 wk, the kidneys of all these animals were perfused with Tris/NaCl and processed for direct immunofluorescence and their sera were tested for anti-brush border activity by direct immunofluorescence and by immunoprecipitation.

Immunofluorescence. Direct immunofluorescence was used for the detection of endogenous IgG in rat glomeruli; 5-μm cryostat sections of Tris/NaCl-perfused, unfixed kidneys were stained with rhodamine-conjugated rabbit anti-rat IgG. Indirect immunofluorescence on unfixed frozen sections of kidneys from normal Sprague–Dawley rats was used for detection of anti-brush border antibodies. Sections were incubated first in the appropriate rat serum; this was followed by staining with rhodamine-conjugated rabbit anti-rat IgG and examination in a Zeiss Photomicroscope II equipped with epi-fluorescence optics. Controls consisted of similar incubations in pre-immune serum, sera from mock-immunized animals, and nonspecific IgG.

Immunoperoxidase. Kidneys were fixed by perfusion in 4% formaldehyde and 0.2% glutaraldehyde (3 min), quenched in 0.1 M glycine, and frozen; cryostat sections were prepared and incubated by an indirect immunoperoxidase procedure as described (17), by using purified IgG from rats with HN induced by FxlA or purified gp330 as the first antibody.

$NaDodSO_4$/Polyacrylamide Gel Electrophoresis. One hundred micrograms of whole microvilli was dissolved in sample buffer (3.65% $NaDodSO_4$/18 mM dithiothreitol/4.5 mM EDTA/6 M urea/10% glycerol). Aliquots (10–50 μl) of column fractions containing membrane proteins solubilized in DOC were precipitated with 0.2 M acetic acid, extracted in absolute ethanol (18), and solubilized in sample buffer. All samples were boiled for 3 min and were subjected to electrophoresis for 24 hr at 7.5–15 mA on gradient (3.6–8%) $NaDodSO_4$ gels (19) with 6 M urea. Gels were fixed and stained in 50% methanol/7% acetic acid with 0.2% Coomassie blue R. β-galactosidase (M_r 116,000) was crosslinked with glutaraldehyde (20), and the dimers and trimers were used as molecular weight markers along with those purchased from Bio-Rad. For autoradiography, the gels were fixed, stained, and dried and then were exposed to Kodak X-Omat RP-5 x-ray film by using a Du Pont Cronex intensifying screen at −70°C.

Immunoprecipitation. Isolated microvilli were radioiodinated (12) and solubilized in RIPA buffer [0.1% $NaDodSO_4$/1% Triton X-100/1% sodium DOC/150 mM NaCl/25 mM Tris·HCl, pH 7.2 (21)/10 mM EDTA]. The lysate was centrifuged at 38,000 × *g* for 20 min, and an aliquot of the supernatant, containing ≈100,000 cpm, was precipitated with 50 μl of serum or 5–25 μg of purified IgG. The following antibodies were used: (*i*) anti-rat FxlA IgG, raised in rabbits; (*ii*) anti-rat FxlA, raised in rats; (*iii*) IgG eluted from isolated glomeruli of rat kidneys with active or passive HN; (*iv*) serum or IgG of rats immunized against isolated gp330; and (*v*) acidic eluates of the isolated glomeruli of the latter animals.

The following served as controls: (*i*) pre-immune sera of rats immunized against gp330; (*ii*) sera of rats immunized against microvillus proteins depleted of gp330; (*iii*) sera or IgG of rats and rabbits immunized with complete Freund adjuvant alone; (*iv*) IgG prepared from sera of rabbits immunized against rat albumin; (*v*) sera of rats immunized against whole isolated microvilli.

Immunoprecipitates of rabbit antibodies were bound directly to Protein A-Sepharose CL-4B (15 mg, preswollen in RIPA buffer). To immunoprecipitate rat antibodies, an intermediate precipitation step with rabbit anti-rat IgG was necessary owing to the low affinity of rat IgG for Protein A. The immunoprecipitates were solubilized by boiling for 3 min in sample buffer and were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis.

Trypsin Treatment of Isolated Microvilli. Isolated microvilli were washed three times in Tris/NaCl without protease inhibitors. Aliquots were digested with TPCK-trypsin (0.1 ml/mg) for 5–60 min at 20°C or 37°C. The reaction was stopped by addition of lima bean trypsin inhibitor (1 mg/ml), the digest was centrifuged at 38,000 × *g* for 20 min, and pellets and super-

natants were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis.

RESULTS

Purity of the Microvillus Preparation. By electron microscopy, the bulk of the pelleted microvillar fractions was seen to consist of individual membrane-limited microvilli, oriented right-side-out, containig a cytoskeletal core. A few membrane-limited vesicles devoid of cytoskeletal elements were also present, and a few structures resembling lysosomes were occasionally encountered at the bottom of the pellet. The specific activity of alkaline phosphatase in typical preparations was ≈13 times that of the starting material.

Protein Composition of Microvillus Membranes. Results obtained by $NaDodSO_4$/polyacrylamide gel electrophoresis of the solubilized microvillar fraction demonstrated the presence of >20 polypeptides (Fig. 1 *Left*, far left lane). The bulk of the proteins was seen at 140 kilodaltons (kDal) or less. One major component banded at ≈330 kDal, based on its mobility as compared to that of the trimer β-galactosidase (M_r 448,000). Another band was seen at 300 kDal, but its amount varied from preparation to preparation.

Immunoprecipitation of Microvillar Components. To identify the antigen present in microvillus membranes responsible for binding of endogenous IgG to the GBM, solubilized ^{125}I-labeled isolated microvilli (Fig. 1 *Left*, lane A) were reacted with (*i*) anti-Fx1A antibodies from the sera of rabbits and rats immunized with Fx1A and (*ii*) IgG eluted from glomeruli of rats with active or passive HN.* IgG obtained from rabbits immunized with Fx1A precipitated seven major polypeptides of the microvillus membrane (Fig. 1 *Left*, lane B), including gp330. Sera from rats with active HN precipitated gp330 as the major band, along with traces of several other polypeptides. IgG eluted from isolated glomeruli of animals with active HN strongly precipitated gp330 and no other peptide (Fig. 1 *Left*, lane C). IgG eluted from glomeruli of rats with passive HN induced with rabbit anti-Fx1A IgG specifically immunoprecipitated gp330. Traces of the 140- and 90-kDal bands (which were the major components of the detergent extracts) were also visible, but they were considered to be contaminants because they were also present in controls in which anti-rat albumin IgG was used as the immunoprecipitant. Pre-immune sera and the sera of mock-immunized rats did not precipitate any protein from solubilized membranes.

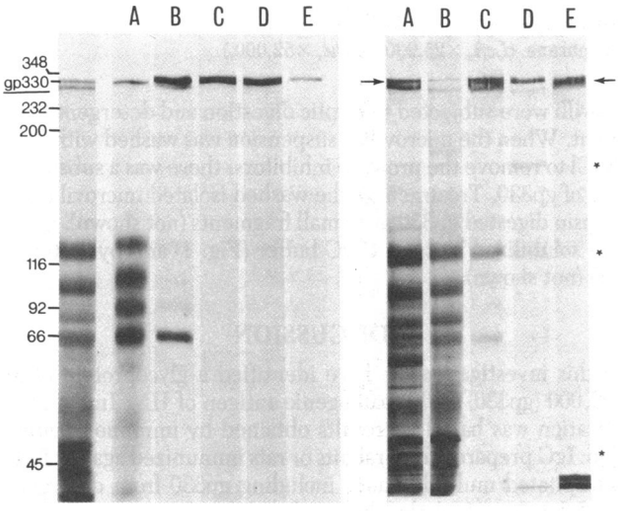

FIG. 1. (*Left*) Autoradiograms of ^{125}I-labeled microvillus proteins extracted by RIPA buffer (lane A) and immunoprecipitates (lanes B–E) obtained therefrom with: rabbit IgG raised against a crude fraction from rat kidney cortex (Fx1A) (lane B); immunoglobulins eluted from isolated rat glomeruli with active HN induced by Fx1A (lane C); serum from a rat immunized against isolated gp330 (lane D); and immunoglobulin eluted from glomeruli isolated from a kidney of a rat immunized against isolated gp330 (lane E). The lane on the far left is a Coomassie blue-stained $NaDodSO_4$/polyacrylamide gel electrophoresis pattern obtained from isolated rat kidney brush borders; protein bands are given in kDal. (*Right*) Coomassie blue-stained $NaDodSO_4$/polyacrylamide gels illustrating the results of the step-wise purification of gp330. Lanes: A, gel pattern of whole microvilli; B, supernatant of microvilli extracted with DOC buffer used as the load for the Bio-Gel A-5m column; C, pooled high molecular weight fractions obtained from the Bio-Gel column which were loaded on a lentil lectin-Sepharose-4B column; D, material released from the lentil lectin column by 20 mM α-methylmannoside; E, material eluted from the lentil lectin column by 500 mM α-methylmannoside/6 M urea/10 mM EDTA, which was used for immunization. The position of gp330 is marked by an arrow. Asterisks mark smaller bands believed to be degradation products of gp330. The bands at the bottom of lane E represent lentil lectin that had been released from the column.

Purification of gp330. The fact that gp330 was the only component of solubilized brush border membrane that was immunoprecipitated by IgG eluted from glomeruli of rats with active and passive HN made it the logical candidate as the pathogenic antigen of HN. Accordingly, an attempt was made to purify gp330. To take advantage of the fact that it had a considerably higher molecular weight than its neighboring proteins on $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 1 *Right*, lanes A and B), gel filtration of solubilized microvillus proteins was performed on Bio-Gel A-5m. The fractions following the void peak contained gp330 as the major component with smaller amounts of lower molecular weight contaminants (Fig. 1 *Right*, lane C). To eliminate the latter, the pooled fractions were bound to lentil-Sepharose-4B. Small amounts of gp330 were eluted with low concentrations of α-methylmannoside (20 mM), along with most of the contaminants (Fig. 1 *Right*, lane D), but the bulk of the gp330 could be released only by a combination of 500 mM α-methylmannoside/10 mM EDTA/6 M urea, indicating that gp330 had an extraordinarily high affinity for this lectin. Analysis of the eluate by $NaDodSO_4$/polyacrylamide gel electrophoresis revealed gp330 and lentil lectin as constituents of this preparation (Fig. 1 *Right*, lane E). The use of inhibitors of proteolysis (pepstatin A, antipain, benzamidine) during microvillus isolation and addition of diisopropyl fluorophosphate to the detergent extract of the membranes increased the yields of gp330 from ≈50 μg to ≈300 μg per 10 mg of solubilized microvillus protein. Degradation of gp330 into smaller fragments (≈100 kDal, 70 kDal, and smaller) occurred on storage, even when the isolation was done in the presence of proteolysis inhibitors.

Induction of HN by gp330. Fischer rats were immunized against purified gp330 to test its pathogenicity. Five of six immunized animals developed antibodies that stained the brush border of proximal tubules by indirect immunofluorescence (Fig. 2 *Lower Left* and *Right*). Moreover, IgG purified from the serum of these actively diseased rats, shown to be monospecific by immunoprecipitation (Fig. 1 *Left*, lane D), stained the luminal aspect of the brush border membranes of proximal tubules in an indirect immunoperoxidase procedure (Fig. 3).

All responding animals also developed granular deposits of endogenous IgG in their glomerular basement membranes (Fig. 2 *Upper*), as tested by direct immunofluorescence. Two of these animals developed large granular deposits, whereas the rest

* Rats were considered to have developed HN when granular deposits of endogenous IgG (active HN) or exogenous IgG (passive HN) were detected in their glomeruli by immunofluorescence.

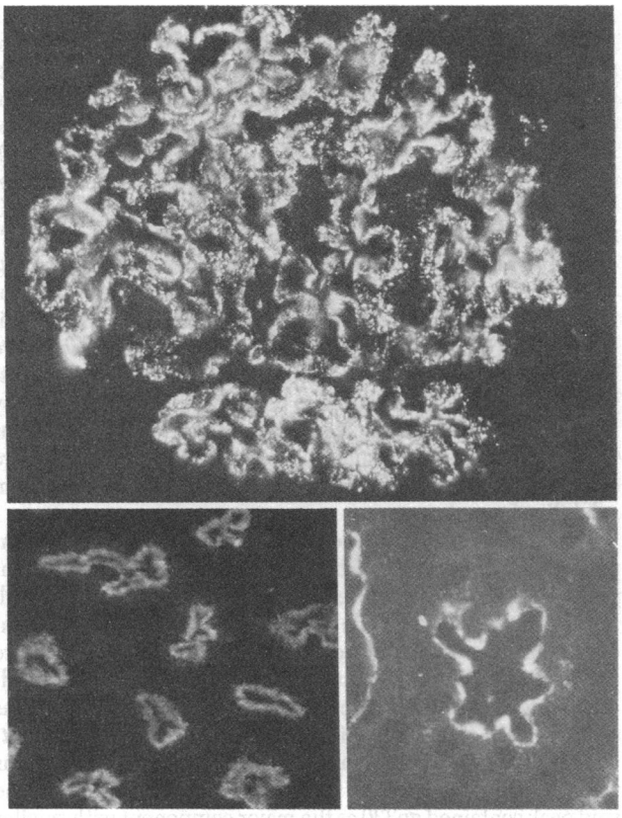

FIG. 2. (*Upper*) Direct immunofluorescence of a glomerulus from a rat immunized against isolated gp330 stained with anti-rat IgG. Endogenous rat IgG is deposited in a granular pattern in the glomerular basement membrane, thus fulfilling the definition of active HN. The deposited IgG has been shown to be monospecific for gp330 (see Fig. 1 *Left*, lane E). (×490.) (*Lower*) Indirect immunofluorescence of a frozen section of normal rat kidney stained with monospecific anti-gp330 prepared from the serum of rats immunized with gp330. The brush borders of the proximal kidney tubules are heavily stained. (*Left*, ×290; *Right*, ×780.)

developed only small tightly packed immune deposits resembling those observed by Edgington *et al.* (2) after a single immunization with a concentrated pathogenic preparation ($Rt_{\alpha}5$). In addition, sera or purified IgG from the diseased rats immunoprecipitated gp330 exclusively, as did IgG eluted from isolated glomeruli of their kidneys (Fig. 1 *Left*, lane E).

When sera or purified IgG from rats with active HN induced by immunization with gp330 was given intravenously to healthy animals, all of them developed the passive disease—i.e., they showed granular deposits of IgG in their glomeruli 3 days after injection of anti-gp330.

Control Experiments. Rats immunized against a suspension of isolated microvilli in complete Freund adjuvant developed active HN as manifest by the presence of endogenous IgG in their glomeruli as previously shown by Miettinen *et al.* (3). Animals that had been injected with complete Freund adjuvant alone did not develop such immune deposits. Rats immunized against a preparation of solubilized microvillar membrane proteins that had been depleted of gp330 by gel chromatography developed antibodies that stained the brush border of proximal tubules by immunofluorescence and immunoprecipitated several membrane proteins from microvilli but not gp330; however, none of these animals developed typical immune deposits in their glomeruli.

Solubilization of gp330. To investigate the nature of the gp330 interaction with the microvillar membrane, isolated microvilli were subjected to tryptic digestion and detergent treatment. When the microvillus suspension was washed with Tris/NaCl to remove the protease inhibitors, there was a substantial loss of gp330. Treatment of the washed isolated microvilli with trypsin digested gp330 into small fragments (not shown). gp330 was solubilized both by DOC buffer (Fig. 1) and by Triton X-100 (not shown).

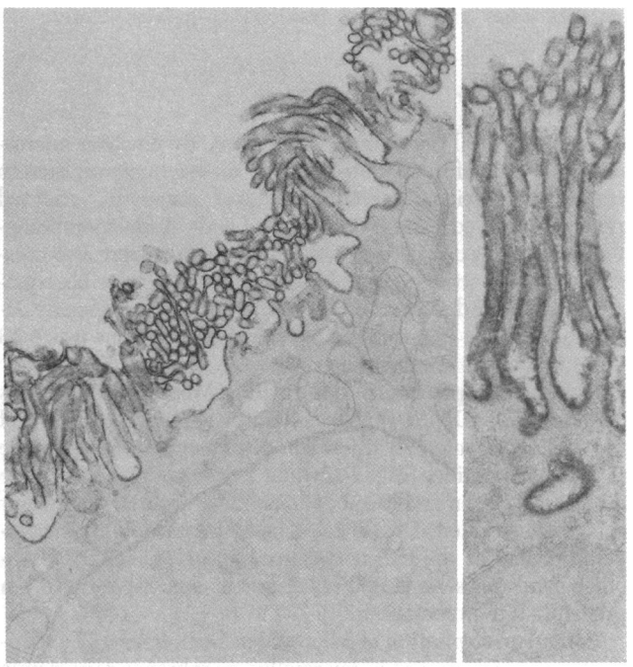

FIG. 3. Normal rat kidney stained with monospecific anti-gp330 rat IgG by indirect immunoperoxidase. Reaction product, which is deposited exclusively on the brush border membranes of the proximal tubule, is seen to be located on the outer surface of the microvillar membrane. (*Left*, ×25,200; *Right*, ×52,000.)

DISCUSSION

In this investigation we have identified a glycoprotein of M_r 330,000 (gp330) as the pathogenic antigen of HN. Initial identification was based on results obtained by immunoprecipitation: IgG prepared from rabbits or rats immunized against FxlA precipitated multiple bands including gp330 from detergent-solubilized, ^{125}I-labeled kidney brush-border microvilli, and, more importantly, antibody eluted from isolated glomeruli of rats with active or passive HN specifically immunoprecipitated gp330. gp330 was subsequently purified by gel chromatography and affinity chromatography on lentil lectin-Sepharose-4B and was used as an immunogen. Rats immunized against purified gp330 developed active HN as evidenced by the deposition of endogenous IgG in a granular pattern in glomeruli. (Recently we also induced active HN with gp330 directly excised from Coomassie blue-stained $NaDodSO_4$ gels.) Moreover, purified IgG prepared from the sera of these animals bound to glomeruli of healthy rats after intravenous injection—i.e., they produced passive HN. These data confirm the identification of gp330 as the HN antigen that leads to the production of monospecific anti-gp330 IgG which binds to glomeruli.

To exclude the possibility that microvillus constituents other than gp330 carry additional pathogenic sites, rats were immunized with solubilized microvillus membrane proteins depleted of gp330 by gel chromatography. Because these animals did not develop active HN we conclude that gp330 carries the sole, or at least the predominant, antigen responsible for this disease.

A number of findings indicate that gp330 is a brush border membrane glycoprotein with its major antigenic determinant(s) facing the tubule lumen: (*i*) it is solubilized from isolated microvilli by detergent treatment; (*ii*) it is released from intact microvilli by trypsin; (*iii*) it is labeled with lactoperoxidase-mediated radioiodination, whereas cytoskeletal core proteins are not (Fig. 1 *Left*, lane A); and (*iv*) it is localized by immunoperoxidase staining on the luminal side of the membrane (Fig. 3). That gp330 is a glycoprotein with exposed mannose groups is suggested by its affinity for lentil lectins, noted by others for the pathogenic principle of HN (3) and by the fact that it specifically binds ^{125}I-labeled concanavalin A (unpublished data).

The protein composition of rat kidney microvilli found in our study resembled that found by Glossmann and Neville (22), who observed sialoglycoproteins of high molecular weight in rat renal brush border membranes by $NaDodSO_4$/polyacrylamide gel electrophoresis. High molecular weight proteins are also present in isolated pig microvilli (23).

Several different approaches have been used to characterize the HN antigen. Edgington *et al.* (2) reported the concentration of pathogenic activity in a large (28.6S) lipoprotein complex, designated RTE_a5, which had been purified from a DOC extract of Fx1A. More recently, Miettinen *et al.* (3) have shown that whole isolated microvilli, detergent lysates, and digests released from microvilli with trypsin induce active HN. Naruse *et al.* (24), who purified a small (8.9S) pathogenic fragment (9), have also shown that the pathogenic antigen is pronase insensitive. The findings that pathogenic activity could be adsorbed from solubilized microvilli by lentil-Sepharose (3) and that brush border immunostaining could be blocked with concanavalin A (8) have been interpreted as indicating the involvement of hexose moieties in the pathogenesis of HN.

The peptides recovered by previous workers were smaller than gp330 (9, 25) and may be fragments of this molecule containing the pathogenic determinant(s), because according to our experience, gp330 seems to be very sensitive to endogenous proteolysis.

The mechanism involved in the selective concentration of anti-brush border antibodies in glomeruli is still debated. For some time it was widely believed to be caused by the deposition of circulating immune complexes (6, 26). More recently, evidence has been obtained by Van Damme *et al.* (27) and Couser and co-workers (28, 29) that suggests that the disease is caused by direct interaction of circulating IgG with antigens present in the glomerular capillary wall. Several attempts have been made to localize the HN antigen in the glomerulus by immunocytochemistry, by using either anti-Fx1A antibodies (30, 31) or IgG eluted from glomeruli of animals with HN (32–34). The selective absorption of monospecific anti-gp330 antibody by the glomerulus as found in this study could indicate the presence of gp330 or crossreactive antigens in glomerular capillaries.

The isolation of gp330 has provided a source of purified HN antigen that has been used to raise monospecific (polyclonal) antibodies and that can be used to raise monoclonal antibodies. The availability of polyclonal and eventually monoclonal antibodies against purified gp330 renders possible the isolation and subsequent characterization of the crossreacting glomerular antigen(s) which is needed to gain further insight into the pathogenesis of this disease.

We thank David Sharkey and Dianna Picton for excellent technical assistance, Pamela Ossorio for preparation of the figures, and M. Lynne Wootton for secretarial and editorial help. This research was supported by U.S. Public Health Service Research Grant AM 17724 (to M.G.F.) and by a fellowship from the Max Kade Foundation (to D.K.). D.K. is on leave from the University of Vienna, Austria.

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